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## ABSTRACT

Five potato (*Solanum tuberosum* L.) leaf cuttings were flown on STS-73 in late October, 1995 as part of the 16-day USML-2 mission. Pre-flight studies were conducted to study tuber growth, determine carbohydrate concentrations and examine the developing starch grains within the tuber. In these tests, tubers attained a fresh weight of 1.4 g tuber<sup>-1</sup> after 13 days. Tuber fresh mass was significantly correlated to tuber diameter. Greater than 60% of the tuber dry mass was starch and the starch grains varied in size from 2 to 40 mm in the long axis. For the flight experiment, cuttings were obtained from seven-week-old Norland potato plants, kept at 5°C for 12 hours then planted into arcillite in the ASTROCULTURE™ flight hardware. The flight package was loaded on-board the orbiter 22 hours prior to launch. During the mission, the flight hardware maintained an environment around the cuttings of 22°C (±2°C), 81% (±7%) RH and a 12 hour photoperiod using red and blue light emitting diodes at a photosynthetic photon flux of 150 μmol m<sup>-2</sup> s<sup>-1</sup>. CO<sub>2</sub> concentration exceeded 4000 ppm during the dark period and was controlled during the light period to approximately 400 ppm. Video downlinking of images of the plants and CO<sub>2</sub> exchange data during the flight demonstrated plant vitality for the first 12 days of the mission followed by senescence of the leaves. The flight package was received 4 hours after landing at the Kennedy Space Center and post-flight processing of the samples was completed within 3 hours. Four out of the five space-grown cuttings produced tubers that were similar in appearance and dimension to the ground control tubers. This is an important finding if potatoes are to be used as part of a bioregenerative life support system for long-term space exploration.

a bioregenerative life support system that would utilize plants to recycle oxygen, carbon dioxide and water as well as provide food. Potatoes have a high yield potential, a high ratio of edible to inedible biomass, and are easy to propagate and prepare for consumption (Wheeler and Tibbitts 1986). In addition, potato tubers are high in digestible starch and provide substantial amounts of protein (Tibbitts and Alford 1982).

It is known that environmental conditions can affect tuber formation. For example, high temperatures inhibit tuber growth and lead to diminished starch content (Krauss and Marschner 1984). As starch represents the major source of calories in potatoes, it is important to understand the factors that might regulate the amount of this carbohydrate in tubers in the spaceflight environment. A number of reports indicate that starch accumulation is reduced in plants exposed to spaceflight (Dutcher et al. 1994, Brown et al. 1996) or to ground-based microgravity conditions such as clinorotation (Brown and Piastuch 1994, Obenland and Brown 1994). The reduction in starch concentrations might result from reduced photosynthesis in space-grown plants (Tripathy et al. 1996), perturbed translocation of carbohydrates throughout the plant (Brown et al. 1996) or altered enzyme activities in the starch synthetic or degradative pathways (Brown and Piastuch 1994, Kordyum 1994). However, existing information is limited on starch concentrations in plant storage organs such as tubers (Kordyum 1994). Therefore the usefulness of potatoes or other starch-storing plant tissues may be limited in a bioregenerative life support system for spaceflight or reduced gravity environments. The studies outlined in this paper will allow us to determine if potato tubers are formed, if starch is stored and if tuber anatomy is altered in the spaceflight environment.

Several workers have shown that excised potato leaves can develop tubers from the axillary bud at the base of the leaf (Gregory 1956, Paiva et al. 1983, Ewing 1985). These tubers accumulate large concentrations of starch. The deposition and structure of the starch in these developing tubers is essentially the same as in tubers developing on stolons on intact plants (Duncan and Ewing 1984). Cell division and enlargement can occur within 24 and 96 hours,

## INTRODUCTION

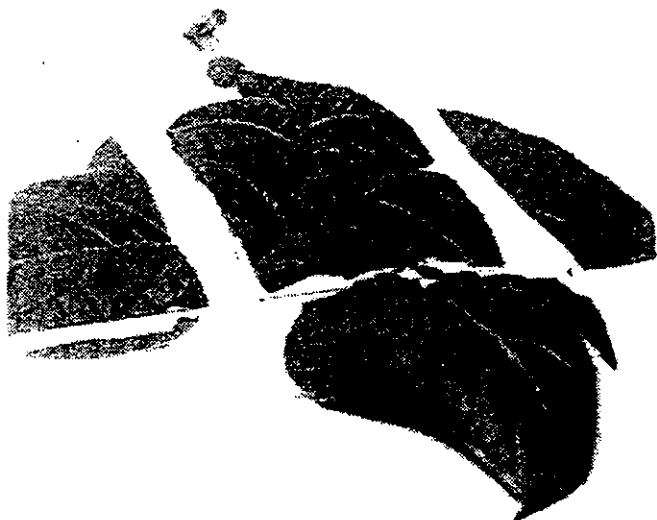
White potatoes are a major source of food throughout the world. The National Aeronautics and Space Administration (NASA) is exploring the use of potatoes as one of the crops in

respectively, after excision of the leaf from the mother plant. Therefore, excised potato leaves capable of forming tubers that fill with starch provide a compact model system for the study of tuber development and metabolism in space (Wheeler 1986).

## MATERIALS AND METHODS

**PLANT PROPAGATION** - Potato (*Solanum tuberosum* L. cv. Norland) plants, started from sterile-culture stem cuttings, were grown in a walk-in growth chamber at the Kennedy Space Center Space Biology Laboratory. Plants were grown in 1 liter plastic containers filled with peat/vermiculite and watered to excess four times daily with a complete nutrient solution (Hammer et al. 1978). Chamber conditions were maintained at 18°C, 70 % relative humidity with a photoperiod of 12 hours light:12 hours dark. The lighting at the plant level was  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux (PPF) and was supplied by cool white fluorescent lamps. After 7 weeks, leaves were excised from selected plants for use in the fifth flight of the ASTROCULTURE™ program (Morrow et al. 1995).

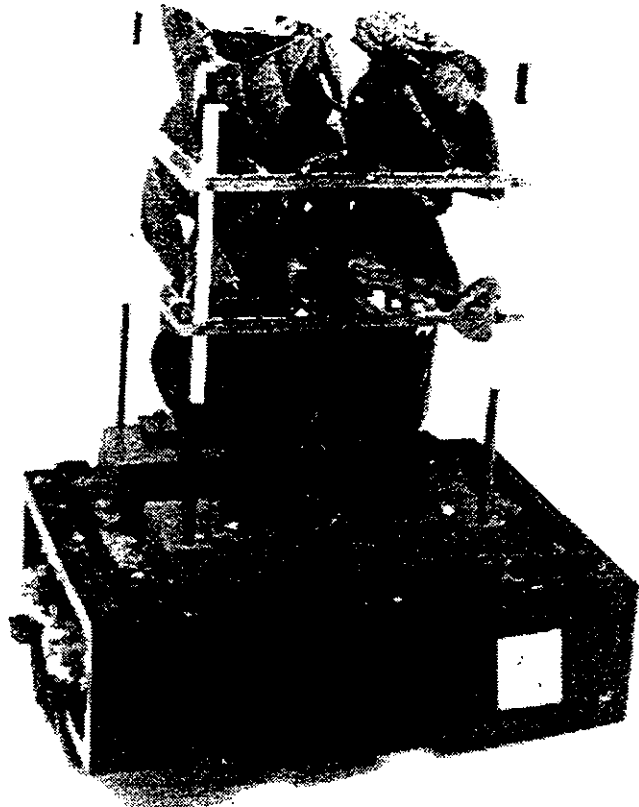
**PRE-FLIGHT PREPARATION** - Three uniform plants were selected to supply flight and ground control leaves. Leaf excision from the mother plants followed the method of Wheeler et al. (1988). The leaves were taken 36 hours prior to the scheduled launch from node positions 7 and 8 counting basipetally from the youngest leaf which was greater than 2 cm in length. A 1.5 cm portion of the stem was included as part of the explant and special care was taken to avoid disturbing the axillary bud. In order to fit within the flight hardware, excess laminar tissue was removed providing a remaining leaflet area of 135 cm<sup>2</sup> (Figure 1).



**Figure 1.** Potato leaf explants prior to insertion into the planting tray.

Leaves were stored in a humid cooler at 5°C until 24 hours prior to the scheduled launch. At that time the cut stumps were gently inserted into pre-moistened arcillite (calcined clay particles) in the rooting tray of the ASTROCULTURE™ flight unit. This unit provided plant lighting using red and blue light emitting diodes, temperature

and humidity control and water delivery using a porous tube system (Morrow et al. 1995). Additionally, the unit was configured for data collection, gas and fluid sampling, CO<sub>2</sub> control, camera observation of the plants and an ethylene removal system. A cover, consisting of closed-cell foam with holes for the petioles, was placed over the planting tray. A total of five leaves fit within the planting tray (Figure 2).



**Figure 2.** Five potato leaf explants inserted into the ASTROCULTURE™ planting tray.

The planting tray was then inserted into the growth chamber housing and the whole chamber assembly was integrated in the flight unit. The unit was transferred to the launch pad and loaded into the Space Shuttle at 22 hours prior to launch. The STS-73 mission, a part of the United States Microgravity Laboratory-2, was launched from the Kennedy Space Center at 10:00 EDT on October 20, 1995. Environmental set points and actual conditions within the plant growth chamber during the mission and ground controls are found in Table 1.

Landing took place at the Kennedy Space Center at 06:45 EST on November 5, 1995 after a mission of 15 days and 20 hours. The hardware was recovered 4 hours after landing and de-integration of the planting tray commenced immediately. Plants were removed from the planting tray, photographed, measured and frozen and/or fixed for later biochemical and/or anatomical measurements.

Mother plants for ground control leaves were started in the walk-in growth chamber at the Kennedy Space Center exactly 4 weeks after the start of the flight mother plants. Leaves were harvested from these plants at 7 weeks after planting and transported to Madison, Wisconsin for the ground control experiment. Ground control leaves were grown in the ASTROCULTURE™ hardware from November 17 through

**Table 1. Environmental control and monitoring capabilities of the ASTROCULTURE™ flight hardware.**

16-day STS-73 Mission				
Parameter	Set point	Ave*	Max**	Min**
Temp (°C)	21	22.1	23.9	21.2
Rel Hum (%)	80	81.3	88.0	76.6
CO <sub>2</sub> (ppm)	500	2308	4100	350

16-day Ground Control				
Parameter	Set point	Ave*	Max**	Min**
Temp (°C)	21	22.0	23.9	20.6
Rel Hum (%)	80	78.2	81.4	75.5
CO <sub>2</sub> (ppm)	500	2102	5758	204

\* Average values for the entire Mission or Ground Control.

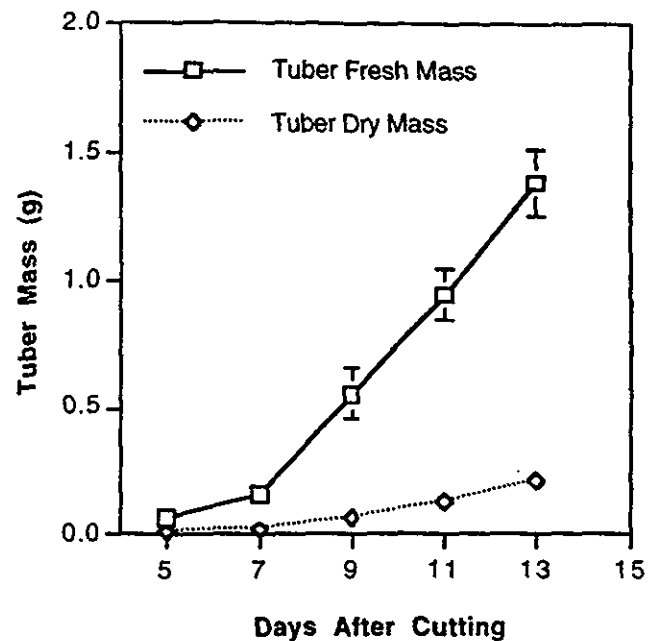
\*\* Maximum and minimum for 12-hour periods.

December 3, 1995. The hardware was maintained in a room of the University of Wisconsin Biotron under temperature conditions duplicating the middeck conditions of the STS-73 mission. Environmental data collected during the spaceflight mission was used to control the plant growth hardware during the ground-control (Table 1). Plant harvest, measurement and tissue preparation occurred exactly as during the postflight harvest.

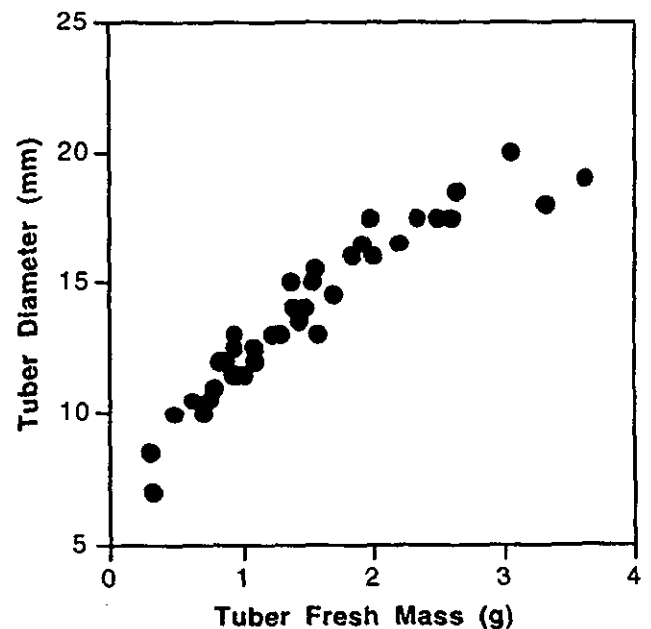
## RESULTS AND DISCUSSION

In order to ensure successful tuber formation in the conditions anticipated during the spaceflight mission, a number of pre-flight studies were conducted. These were ground-based studies using plants grown in a similar fashion to the ones described for the flight experiment.

**TUBER GROWTH** - Tuber size, measured as fresh mass or dry mass, typically increased in a linear fashion between 7 and 13 days after excision from the mother plants (Figure 3). In all studies, tuber diameter was significantly correlated to tuber fresh mass (Figure 4). These results indicated that adequate tuber formation and growth occurred within the time-frame of the proposed flight experiment and that estimates of tuber mass could be achieved through non-destructive measurements of tuber diameter.



**Figure 3. Growth of tubers in axils of potato leaf cuttings.** Values represent the means of 9 replicates and the standard error is shown.



**Figure 4. Fresh weight as a function of diameter of developing potato tubers.**

**TUBER AND LEAFLET CARBOHYDRATE MEASUREMENTS** - It was necessary to ascertain if the potato explants and tubers would contain starch and soluble carbohydrates of measurable quantities when cultured under conditions anticipated for the mission. Leaf cuttings were cultured in a arcillite rooting matrix and harvested after 14 days. Laminar, stem and tuber tissues were frozen in liquid nitrogen, freeze-dried and analyzed for starch, sucrose, glucose

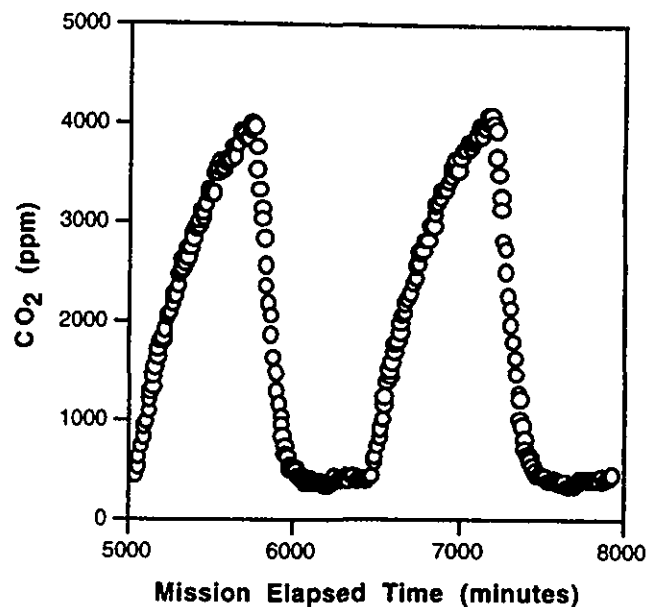
and fructose as in Brown et al. (1994). Quantifiable concentrations of these carbohydrates were present in all the tissue types (Table 2).

**Table 2. Carbohydrate concentrations in laminar, stem and tuber of 14-day old potato leaf explants. Values represent the mean of 6 replicates and the standard error is shown in parentheses.**

	Concentration (mg (g dry weight) <sup>-1</sup> )		
	Laminar	Stem	Tuber
Starch	9 (2)	74 (12)	634 (34)
Sucrose	17 (3)	25 (2)	32 (9)
Glucose	28 (6)	34 (5)	3 (1)
Fructose	1 (1)	1 (1)	0 (0)

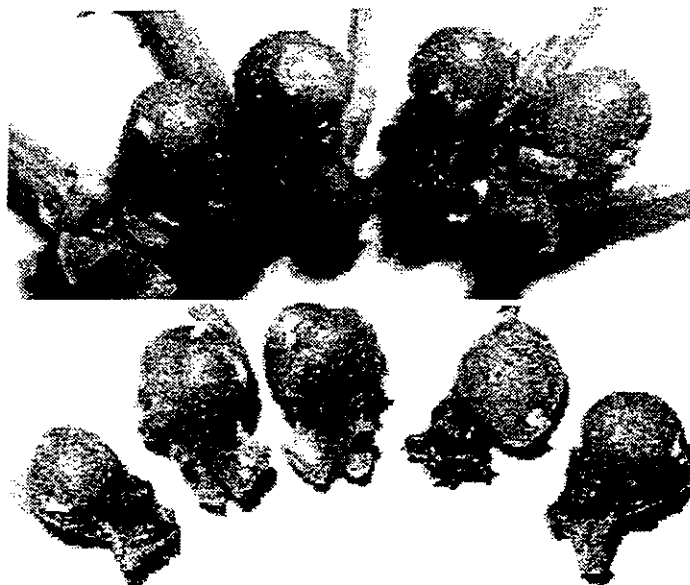
High levels of starch were present in tuber tissue with 8.5-fold and 70-fold lower concentrations in the stem and laminar tissue, respectively. Concentrations of the carbohydrate fractions were similar in the tissues of leaves from positions 5 through 8 counting basipetally from the apex of the mother plant (data not shown). These concentrations were easily measured and were comparable with previous studies (Hannapel 1991). The extremely low concentration of fructose in the tuber tissue is consistent with the observation that fructose is a poor precursor for the synthesis of starch (Hori 1954). These results indicated that measurable quantities of carbohydrates were present in all tissue types under the cultural conditions anticipated during spaceflight and that the choice of leaf position did not affect carbohydrate concentrations during subsequent tuber formation. Starch grains were measured in tubers from 14-day-old plants. The grains ranged in size from 2 to 40 mm in the long axis. The smaller grains were spherical and the larger grains were eccentric.

**IN-FLIGHT MEASUREMENTS** - During the STS-73 mission, concentrations of CO<sub>2</sub> in the growth chamber were periodically downlinked from the Space Shuttle to researchers on the ground. Measurements for one 48 hour period are shown in Figure 5. The reduction in CO<sub>2</sub> during the light periods (5800 - 6520 min and 7240 - 7960 min) indicated that the plants were photosynthetically active during this period. This pattern was consistent until day 12 when there was a marked senescence of the leaves. These data, in combination with downlinked video images of the plants, indicated that the plants were healthy and fixing carbon for the majority of the mission.



**Figure 5. CO<sub>2</sub> concentrations within the plant growth chamber from mission elapsed time 84 hours (5040 min) to 132 hours (7920 min).**

**POST-FLIGHT MEASUREMENTS** - Tuber formation and growth took place in the spaceflight grown tissue (Figure 6, top). The size and appearance of the space-grown tubers were not significantly different from the ground control tubers (Figure 6, bottom).



**Figure 6. Potato tubers formed after 16 days in space (top) and ground control tubers (bottom). Scale is 1:1.**

Excluding the tuber on the far left in Figure 6, top, the mean diameter of the space-grown tubers was not different from the ground-control tubers, indicating the tuber fresh mass may not be significantly impacted by the space environment (Figure 4). These results indicate that spaceflight is not an impediment to tuber formation. This is an important finding if potatoes are

to be used as part of a bioregenerative life support system for long-term space exploration (Wheeler and Tibbitts 1986). The effects of the spaceflight environment on the composition and structure of starch and other components of the potato tuber will be defined upon completion of the biochemical and anatomical analyses.

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## REFERENCES

Brown, CS and WC Piastuch 1994. Starch metabolism in germinating soybean cotyledons is sensitive to clinorotation and centrifugation. *Plant, Cell and Env.* 17:341-344.

Brown, CS, BC Tripathy and GW Stutte 1996. Photosynthesis and carbohydrate metabolism in microgravity. In: Plants in Space Biology H Suge and H Takahashi (eds). Publisher (in press).

Duncan, DA and EE Ewing 1984. Initial anatomical changes associated with tuber formation on single-node potato (*Solanum tuberosum* L.) cuttings. *Ann. Bot.* 53:607-610.

Dutcher, FR, EL Hess and TW Halstead 1994. Progress in plant research in space. *Adv. Space Res.* 14(8):159-171.

Ewing, EE 1985. Cuttings as simplified models of the potato plant. In: Potato Physiology PH Li (ed). Academic Press, NY, pp. 153-207.

Gregory, LE 1956. Some factors for tuberization in the potato plant. *Amer. J. Bot.* 43:281-288.

Hammer, PA, TW Tibbitts, RW Langhans and JC McFarlane 1978. Base-line growth studies of 'Grand Rapids' lettuce in controlled environments. *J. Am. Soc. Hort. Sci.* 103:649-655.

Hannapel, DJ 1991. Characterization of the early events of potato tuber development. *Physiol. Plant.* 83:568-573.

Hori, S 1954. Formation of storage starch in storage organs. I. The starch formation in potato tuber. *Bot. Mag.* 67:57-62.

Kordyum, EL 1994. Effects of altered gravity on plant cell processes: results of recent space and clinorotation experiments. *Adv. Space Res.* 14(8):77-85.

Krauss, A and H Marschner 1984. Growth rate and carbohydrate metabolism of potato tubers exposed to high temperatures. *Pot. Res.* 27:297-303.

Morrow, RC, NA Duffie, TW Tibbitts, RJ Bula, DJ Barta, DW Ming, RM Wheeler and DM Porterfield 1995. Plant response in the ASTROCULTURE flight experiment. *SAE Tech Paper* #951624.

Obenland, DM and CS Brown 1994. The influence of altered gravity on carbohydrate metabolism in excised wheat leaves. *J. Plant Physiol.* 144:696-699.

Paiva, E, RM Lister, WD Park 1983. Induction and accumulation of major proteins of potato in stems and petioles. *Plant Physiol.* 71:161-168.

Tibbitts, TW and DK Alford 1982. Controlled ecological life support system use of higher plants. *NASA Conf. Pub.* 2231. Moffett Field, CA.

Tripathy, BC, CS Brown, HG Levine and AD Krikorian 1996. Growth and photosynthetic responses of wheat plants grown in space. *Plant Physiol.* 110:801-806.

Wheeler, RM 1986. Potato leaf explants as a spaceflight test system. *NASA TM* 88215.

Wheeler, RM, DJ Hannapel and TW Tibbitts 1988. Comparison of axillary bud growth and patatin accumulation in potato leaf cuttings as assays for tuber induction. *Ann. Bot.* 62:25-30.

Wheeler, RM and TW Tibbitts 1986. Utilization of potatoes for life support systems in space. I. Cultivar-photoperiod interactions. *Am. Pot. J.* 63:315-323.